Molecular Marker Based Diagnostics for Almond Bud-Failure

Project No.: **11-HORT7-Gradziel**

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Objectives:

A. Consolidate historical and recent data from almond x almond as well as almond x peach breeding populations for evaluation of possible inheritance patterns of bud failure (BF) in progeny.
B. Develop genetic/epigenetic model(s) based on compiled progeny segregation and development patterns and current research on similar genetic/epigenetic afflictions.
C. Initiate a preliminary assessment currently available molecular-based diagnostics for discriminating between high and low-BF expression.
D. Publish results from BF-heritability studies as a basis for a subsequent proposal to a granting agency targeting molecular-based BF predictors.

Interpretive Summary:

Previous UC Davis (UCD) studies have led to an understanding of the pattern of Non-infectious Bud-failure (BF) development within commercially important almond clones, allowing the effective selection of clonal sources with lower probabilities of expressing BF during the crucial early years of orchard growth (Ref. 4, 12, 19, 20). Earlier attempts at the development of molecular-marker based diagnostics of BF-potential however, were not successful, presumably because the genetic deterioration is not associated with changes in the marker-targeted DNA sequence of the gene(s) involved, but rather involves a suppression of gene activity through, still poorly understood epigenetic mechanisms (4, 8, 18). As a result, our research program has pursued the capability for a range of genetic (3, 4, 6, 14, 16), cytogenetic (9), genomic (1, 5, 8), and proteomic (17) diagnostics while concurrently identifying genetic populations to facilitate genomic/epigenetic analysis. This project consolidates and assesses our current BF inheritance data with eartly results from molecular marker diagnostics in the context of these emerging models of epigenetic control with the goals of: 1) identifying potentially useful developmental models, genetic models, and related molecular diagnostics for this disorder; and 2) leveraging this extensive almond BF knowledgebase as a valuable model system of epigenetic disorders in plants, and thus attracting more extensive outside research funding.

Introduction:

Non-infectious Bud-Failure (BF) remains a major threat to almond production in California, particularly with the recent rapid expansion of acreage. It is a particularly serious problem for the commercially important cultivars Nonpareil and Carmel, which together make up approximately 50% of total plantings. Clonal selection of low BF sources has allowed continued plantings of both Nonpareil and Carmel after BF first became a problem in these cultivars. However, BF-potential (which is related to the age and propagation history of the cultivar) in even the best clonal sources of Carmel may not be sufficiently low to ensure continued commercial use. Careful selection of low-BF Nonpareil clones in the 1970s, 80s and 90s has allowed continued plantings of this dominant variety, though recent BF expression in some Nonpareil sources caution that they may also be progressing towards a new round of BF expression. High BF expression was also a major contributor to the early abandonment of otherwise very promising cultivars such as Merced, and will likely be found in some of the recently released California varieties, particularly those which have the BF-susceptible cultivar Nonpareil as a parent (which includes virtually all currently commercially important cultivars).

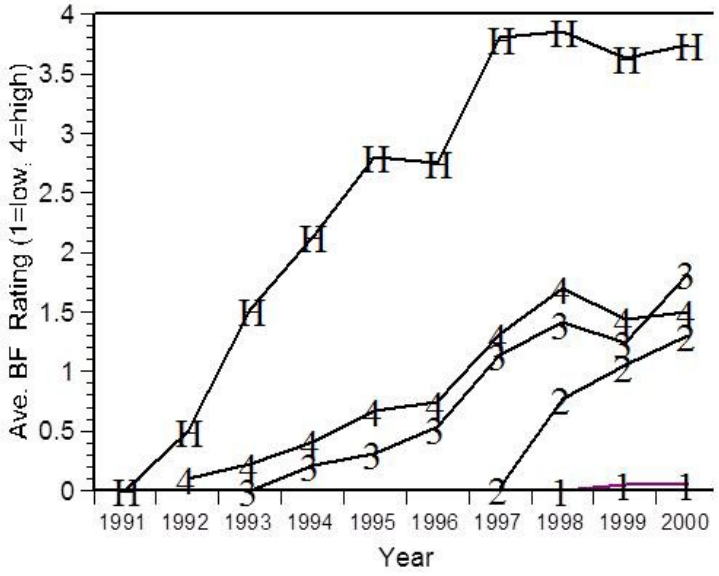


Figure 1. Development of BF- expression in vegetative progeny of different clonal sources of Carmel: 1-original Carmel seedling tree; 2-standard low-BF FPS 1 source; 3-medium-BF FPS 2 source. (Line 4 and Line H are other Carmel cloned that are not covered in this report.)

BF-like symptoms have been observed in isolated trees of some recent releases including the cultivar Winters. Molecular marker analysis has verified the Winters identity but the source of the budwood was not virus-free FPS foundation stock but was probably propagated from virus infected wood gathered from the early Delta research block trials. Similarly, BF-like 'crazy- top' shoot growth was also observed in Marcona trees recently planted in the southern San Joaquin valley. ELISA analysis however showed the symptoms to be the result of Prunus Necrotic Ringspot virus infection. While BF has been shown to be inherited in progeny, the genetic control of BF remains elusive.

Populations which should segregate for BF-expression have been developed from crosses of almond selections to high-BF Nonpareil clones (to asses BF-potential among clones of the same variety), as well as by crosses of almond varieties early-flowering peach genetic-tester lines (to asses latent BF-potential among different varieties). Resultant inheritance data is being used to establish and test different genetic and molecular models for BF. Sequence

data for BF-positive and BF-negative Nonpareil trees is currently being analyzed as is software with the potential for searching for possible molecular markers.

Results and Discussion:

Bud-failure characterization.
Farm calls over the course of this project have typically identified multiple and distinct causes of shoot bud-failure in almond;

- Nutrient deficiencies/toxicities
- Variety growth habit
- Low winter chilling
- Wind rubbing
- Virus/viroids
- Bacterial? Bud-drop
- Noninfectious Bud-Failure (BF) (also known as Crazy Top)

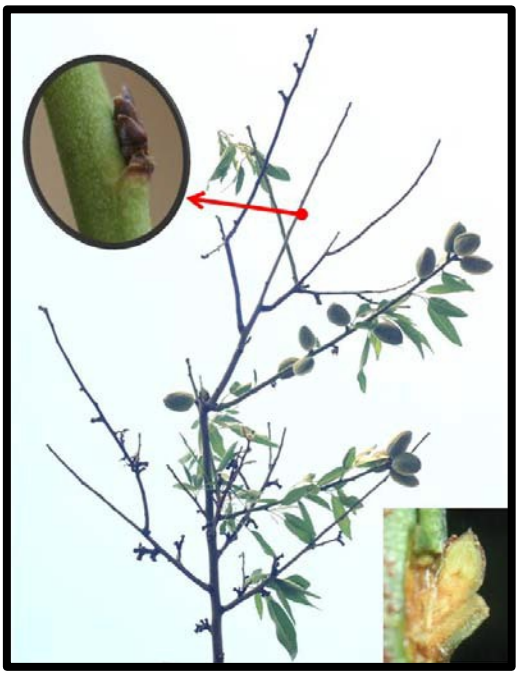


Figure 3. Characteristic shoot development pattern of noninfectious bud failure resulting from a seasonal pattern of die back and regrowth. Lower inset shows the characteristic die back of buds the previous fall with no further development of buds through the winter and following spring (upper inset).

True noninfectious bud failure is characterized by the death of terminal or sub-terminal shoot buds during the previous Fall, which can be verified by a brown necrosis of the internal bud tissue at that time (see insets in **Figure 2**) as well as failure of all subsequent bud swelling and development during the subsequent winter and spring. The disorder becomes evident with the failure of the buds to grow the following Spring resulting in sections of blind or bare shoot-wood and the subsequent pushing of the still-viable basal vegetative buds. Flower buds are not affected and can often developed into fully formed nuts despite the lack of any nearby vegetative leaf growth. A third distinct BF characteristic is that once bud-failure symptoms develop, normal growth is not restored in subsequent seasons but rather the disorder gets worse with each following season (though the extent and rate of shoot failure may vary in subsequent years depending upon growth rate, heat stress from the previous summer, etc). This recurring sequence of terminal shoot-bud failure and pushing of a viable basal buds results in a punctuated and erratic shoot development pattern commonly termed "crazy top" (**Figure 2**). In some severe cases of BF, the bark on young shoots can develop a characteristic splitting or cracking often called ‘rough bark’ (**Figure 3**). BF is ‘noninfectious’ i.e. it cannot be transmitted to other trees by budding or grafting.

Figure 2. ‘Rough-bark’ trait sometimes observed in severe noninfectious bud failure.

In contrast, bud-failure from nutrient deficiencies/toxicities (including some herbicide toxicities) often show some bud development during the winter chilling period and subsequent spring growth, as is the case with zinc-deficiency in **Figure 4a**. Leaf and shoot appearance is often

characteristic of the specific toxicity/deficiency. Normal growth can also be restored with the proper nutrient treatment.

Similarly, some varieties such as the late-blooming variety Savanna (**Figure 4b**) show a late leafing-out on terminal shoots that give an early impression of BF. Close examination of shoots, however, typically showed buds are developing although at a delayed rate. This can also be confirmed by revisiting the orchard one to two weeks later when normal shoot development should be observed.

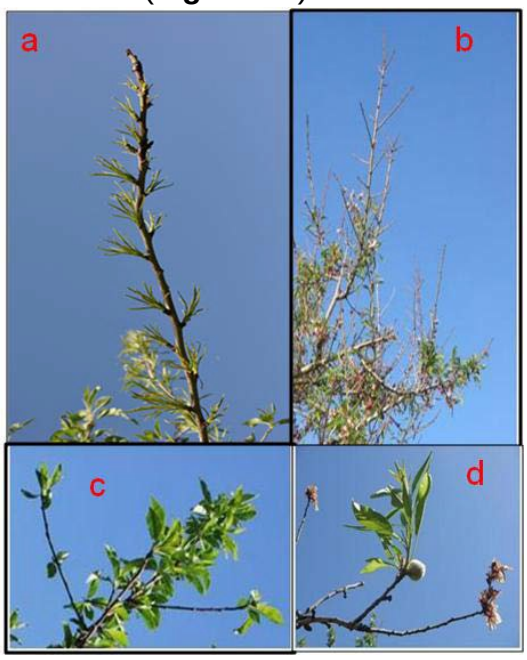


Figure 4. Expression of bud failure from different biotic and abiotic agents.

In years with low winter chilling, some varieties, including Carmel, may also show a delay in terminal or subterminal lateral bud development (**Figure 4c**). Again, a close examination of the buds will show some degree of swelling or development from the previous fall, ruling out noninfectious bud failure. As with late blooming varieties, buds may continue development at a later date though in some cases they appeared to become dormant or even desiccated. A similar appearance is sometimes caused when shoots or branches rubbed together in the wind causing the sloughing of buds. Close examination of the shoots can often identify the physical damage from rubbing as well as the responsible branch.

A form of bud failure often observed on old to very old trees is infectious bud failure, or bud failure caused by virus infection (typically Prunus Necrotic Ringspot Virus or Prunus Dwarf Virus). Where noninfectious bud failure will typically first appear in the rapidly growing shoots at the tops of trees, infectious bud failure tends to be more prevalent at the slower growing shoots on the trees lower branches. New shoot growth tends to show shortened internodes and be willowy giving a 'mules-tail' appearance (**Figure 4d**). Flowers may or may not be affected depending upon the virus and variety. Diagnosis of infectious bud failure is by graft or bud transmission to a susceptible host, or by ELISA or molecular analysis (see **Appendices A** and **B**).

Models for Noninfectious Bud-Failure development. In our evolving model of BF, the critical Fall bud degeneration results from the deterioration in function of gene(s) vital to vegetative bud transition to winter dormancy. This deterioration results from a gradual genetic 'ageing' of a crucial gene complex as a consequence of repeated phase cycling of meristematic cells. Such cycling occurs during the yearly growth phases of almond shoots and appears to also occur,

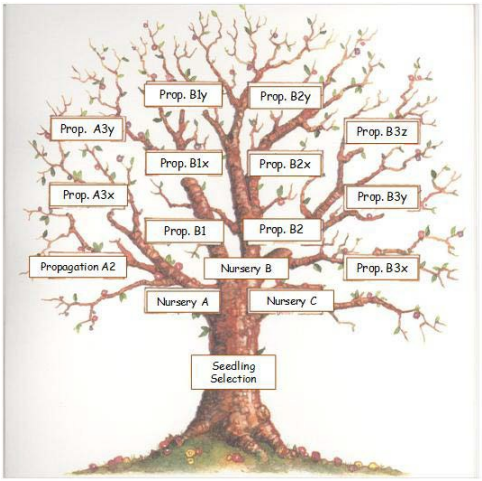


Figure 5. Tree model for the increase in potential for BF appearance either in an orchard tree or (analogously) nursery propagation sources.

and may even be amplified, by vegetative propagation. The typically ramified propagation history of most vegetatively propagated tree crops is thus analogous to the growth and development of a mature tree (**Figure 5**). Since BF appears to be determined by an 'internal aging' process, the appearance of BF symptoms at the terminus of one branch (clonal propagation source) is a good predictor of imminent BF appearance on other branches (propagation source) independently ramifying from a common source. Genetic deterioration also appears to be correlated with environmental stresses, particularly heat, during early-season bud development to summer dormancy (4, 20). Low BF-potential propagation sources have been selected from among clonal lines in which gene ageing is limited owing to their lineage (recent line of descent from original cultivar seedling tree) and previous growth environment (including low heat stress and propagation method) (12). Such vegetative progeny based clonal studies, however, typically require 10 or more years to accurately characterize clonal-source BF-potentials. A well-characterized example of this approach was the selection in the 1990s of Carmel clonal nursery sources which showed lower potential for developing BF symptoms when used as propagation material (**Figure 1**). Significantly, even the best sources showed symptoms within the first 10 years of tree growth showing that while the BF potential could be reduced dramatically, it would still be a concern even in the most promising propagation sources (particularly since an additional 2 vegetative generations of ageing {i.e. mother block and grower trees} are required prior to commercialization). This clonal-source selection as applied to Carmel was originally applied to Nonpareil when BF symptoms became particularly problematic in the 60s, 70s and 80s. To, in a sense, turn back the internal-aging clock, epicormic buds from the base of old Nonpareil trees initially planted in the early 1900s were pushed to develop shoot growth from which clonal source material was propagated (**Figure 7**). Because the Nonpareil cultivar originated in the 1880s, these basal epicormic (i.e. poorly differentiated) buds from old trees would represent relatively low BF potentials (because they were laid down early in tree growth and remained largely dormant in the intervening years). As such, they would serve as good foundation material for continued Nonpareil propagations. That it took approximately 50 years for Nonpareil to initially show BF-symptoms indicates that the original seedling selection had relatively low initial BF potential. However, while low BF-potential was recovered from trees

2011 Data	DELTA	KERN	FPMS
(Proportion of trees of	different clonal	sources	showing BF)
CARMEL#1			
3-56-1-90	7%	26%	-
NONPAREIL			
3-8-2-70		9%	-
3-8-6-72		7%	-
3-8-5-72	-		
3-8-8-72	-		-
3-8-16-90			-
3-8-12-72			-
3-8-18-92			

Figure 6. BF.-expression in an initially low BF-potential UCD Nonpareil source showing recent BF occurrence in previously clean material.

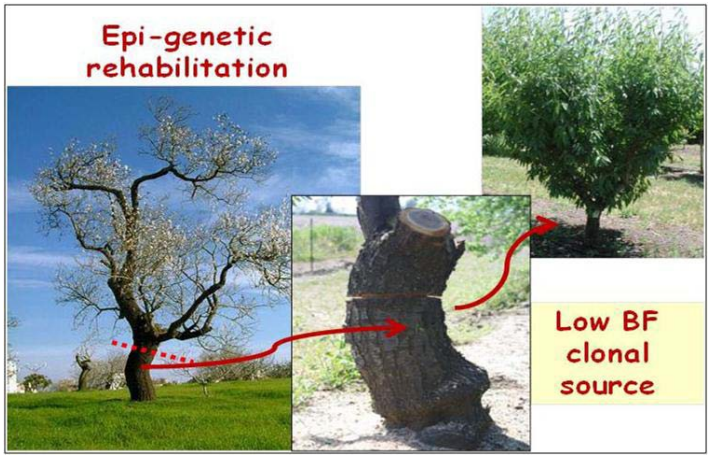


Figure 7. Rehabilitating Nonpareil almond to a lowered BF status by propagating new nursery foundation blocks from BF-dormant basal epicormic buds pushed from 100-year-old trees**.**

planted in the early 1900s, their BF-potential would be expected to gradually age (decay) in the ensuing 50 years to the point that BF-expression is again becoming a problem.

Evidence of such low BF-potential erosion has recently been observed in a Nonpareil-clonal source originally identified for low BF-expression/BF-potential (**Figure 6**). While increasing levels of BF-expression are expected in relatively young (20 years) clonal sources of Carmel because of its higher initial (seedling tree) BF potential, it has not been previously observed in the generally more durable low BF-potential Nonpareil clonal sources selected in the 70s and 80s. The commercially important IR2 Nonpareil selection (3-8-2-70) was selected at a similar time and from similar material as the other industry important sources, Jeffries and McEnespy. BF expression in Nonpareil trees from this and related lineages has recently been documented (**Figure 6**). Data in **Figure 6** was developed from 20 plus year-old orchards of these initial clonal sources which are still present in some Sacramento and San Joaquin Valley locations. Consequently, the BF expression levels serve as in indication of the BF-durability of these different sources. Southern San Joaquin Valley locations (Kern County in **Figure 6**) consistently give some of the best assessments of long-term BF-durability (see Citation 12) because of the generally greater heat stress. [Interestingly, the IR2 (3-8-2-70) Nonpareil clone also shows some of the highest levels of cumulative production in recent San Joaquin regional trial studies by Bruce Lampinen et al. (**Appendix C**).

Variety	DELTA	KERN	FPMS	Grower
Aldrich	-	-	-	
Butte	-	-	-	
Chip's	-	?		
Donna	-	-		
Fritz	-	-	-	
Jenette	-	X		
Jiml	-	-		
Johlyn	-	?		
Kahl	-	?		
Kaperiel	-	-	-	
Livingston	-	-		
Milow	-	-	-	
Mission	-	-	-	
Monterey	-	-	-	
Morley	?	-		
NPU	-	-	-	
Padre	-	-	-	
Peerless	-	-	-	
Plateau	-	-		
Price	-	-	-	
Rosetta	-	-	-	
Ruby	-	-	-	
Sano	?	-		
Savana	?	-		
Sonora	-	-	-	
Wood Colony	-	-		
Yokut	?	X		
Winters	-	-		X
2-19E	-	-	-	

Figure 8. Results from 2010-12 BF surveys at the Delta and Kern Regional Variety Trials as well as local grower trials and FPS foundation sources.

While careful selection in the 50s and 60s of source material based on BF-expression (as determined using both such vegetative progeny tests and the more rapid test-crosses method described below), allowed continued production of low BF Nonpareil trees, even these more elite lines are beginning to show BF again. Reduced BF-expression is also facilitated by carefully selection of those propagation lineages remaining free from BF-expression or returning to the original 1950s selections (where available). As part of this project, new FPS parent clonal stock were established via such basal epicormic buds rehabilitation (**Figure 7**) for the Nonpareil sessions (3-8-5-72) , (3-8-2-70), (3-8-8-72) and (3-8-16-91) and Carmel accession 3-56-1-90.

Figure 9. BF-like symptoms on Winters trees in Fresno County in 2010-11**.**

Several recent varieties such as Yokut, Kochi and Jenette also appear to show evidence of early BF expression (**Figure 8**). However, since plantings of these varieties are not expected to be commercially significant, the evaluation/selection of low BF-potential sources may not be warranted. A single case of potential BF in the more commercially important cultivar Winters has been identified in eastern Fresno County (**Figures 8 & 9**). The low number of trees showing symptoms also showed growth habits somewhat inconsistent with the Winters variety. Molecular analysis of leaf samples collected from these trees, however, has verified that they are the cultivar Winters (**Appendix B**). Winters has been known to be vulnerable to BF based on both lineage (it has the BF-affected cultivars Nonpareil, Harriet, and Jordanolo as parents, see **Figure 12**), however, from BF test-crosses [in an earlier Winters x high BF Nonpareil cross, progeny showed a low proportion of bud failure trees indicating a low BF potential]. The low potential for Winters was comparable to Sonora, which gave similar progeny test results and despite its extensive plantings has only shown the occasional BF tree). A more recent and more accurate test of BF potential involves the control crossing with an early flowering peach tester stock (UCD 4A-17) as described below in Genetic/Epigenetic Models. Results (described below) support an existing but low BF potential for the Winters cultivar. In addition, the bud-wood source used to propagate the early Fresno County test block trees where BF was observed was not from the established FPS foundation source, but was traced back to very early test plantings in the Stockton area which were later found to be virus-infected.

Figure 10. Bud-failure in the Marcona almond variety resulting from Prunus Necrotic Ringspot Virus infection.

BF-like symptoms have also been observed in Southern San Joaquin Valley Marcona plantings (**Figure 10**). Molecular (ELISA) analysis, however showed the symptoms to be the result of virus induced bud-failure, in this case due to infection of Prunus Necrotic Ringspot Virus (PNRSV in **Appendix A**). The virus was also verified through graft-transmission (work done in cooperation with FPS labs). Extensive virus testing of different Marcona source material has identified a single tree source which has been shown to be negative for both Prunus Necrotic Ringspot Virus and Prunus Dwarf Virus (**Appendix A**). This clonal source material has now been transferred to FPS foundation stock plantings and is undergoing final trueness-to-type testing.

Genetic/epigenetic models and associated molecular-based diagnostics.

Different genetic control models, including control by 1 to 3 Mendelian-type genes, as well as various epigenetic mechanisms are consistent with observed segregation patterns (**Figure1 & 11**). In almond by almond crosses, the possible interaction between functional and non-functional forms of the BF gene(s)

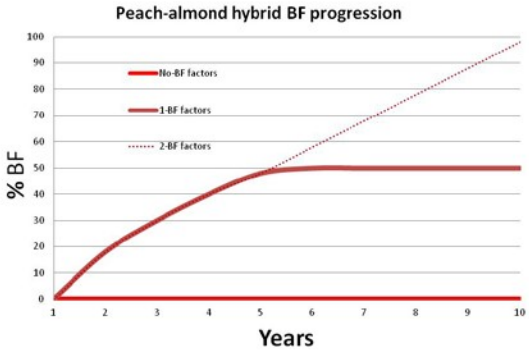
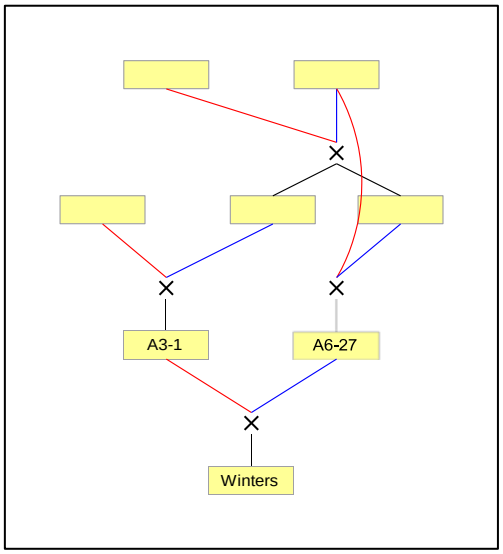


Figure 11. Expected peach by almond progeny performance when the almond parent contains one or two high BF-genes forms.

is possible because each parent will contribute a genetic factor and the presence of a functional factor may act to mask the presence of a nonfunctional BF-factor.

Previous work with almond by peach interspecies hybrids, (**Figure 11**), however, has demonstrated that the very early flowering peach tester (UCD40A-17) appears to lack a BF-type gene and so would not act to mask any aberrant BF-gene expression of the almond parent tested. With no homologous BF-functional gene to mask the expression of BF-expressing genes, progeny should show BF-symptoms when BF-forms of the gene are present. Because BF-factors would be inherited entirely from the almond parent, the performance of the peach by almond progeny could be used to determine the almond parent genotype as shown in **Figure 11**. If the almond parent contained no BF-inducing factors/genes then no progeny would show BF (solid basal red line in **Figure 11**). If the almond parent had one BF factor and one normal factor than only half the progeny would be expected to eventually show BF (curved rust line in **Figure 11**). If both factors/genes in the tested almond parent were BF then all progeny would be expected to eventually show BF (dotted line in **Figure 11**). Thus progeny performance can identify the BF-potential of almond parents even when no BF has previously been observed in those parents, though the test requires several years for completion. In addition, data from earlier studies suggest that the strength of BF-potential in the almond source will be correlated with the rate of BF expression in the seedlings and the final level of BF expression in individual seedlings. Thus, while test-cross progeny from an almond x high-BF almond cross in are useful in identifying low-BF sources within the same clone, test-cross progeny from almond x early-flowering peach testers are useful in the early identification of general BF-potential of new breeding selections and varieties such as Winters. We are currently in the third year of progeny testing from a Winters by UCD40A-17 test cross. Of 25 individuals in the population, none has shown bud failure to date though according to the peach-almond gene model, approximately 30% of the individual should be showing bud failure. Similar results have also been obtained with Sonora and other well-established almond cultivars such as Peerless which have occasionally showing bud-failure symptoms, but only in isolated instances. Because of Winters unique and well-established lineage (**Figure 12**) and it's having both the high-BF Nonpareil and Jordanolo as parents, this high-BF almond variety as well as high-and low-BF Nonpareil clones and breeding selections are being further analyzed using high-resolution genetic mapping (**Appendix D**). Association mapping procedures can then be used to identify certain genetic combinations in progeny which are always associated with BF expression. These genes might then be used as markers (since their association with that trait indicates they are closely linked to the causative gene) as well as a starting point to identify the specific causative gene. Towards this goal, several hundred progeny from a high-BF Carmel by UCD40A-17cross in



which progeny are expected to strongly segregate for BF (based on previous performance), have been generated in 2011-12. The presence and extent of BF in individual progeny trees will be rated based on criteria developed in literature (see citation12). Information on the time that BF was first observed in individual progeny trees will also be included in the database. The rate of BF progression in both individual trees as well as in the combined progeny population will be evaluated as a possible predictor of BF-potential of the almond parent variety. Inheritance models supported by this preliminary data will then be evaluated. Previously established genetic relationships (see citations 3, 4, 8) among almond varieties tested will also be considered when evaluating inheritance models.

Standard genetic dogma states that a trait such as BF results from the action of a specific protein controlling a specific plant developmental process. Since the specific protein structure is coded for by a unique sequence of DNA (gene), the definitive marker for that trait is the DNA sequence coding for the controlling protein. This model has proven successful in describing and genetically manipulating numerous processes in plant development and has led to a proliferation of accurate DNA-based molecular markers for many traits. BF is a genetic disorder in almond which is expressed as a failure of vegetative bud growth leading ultimately to tree decline. Current data indicates that BF does not fully follow the standard genetic model but rather is due to the failed expression of a gene/gene complex required for normal growth and development. In this case the DNA sequence (gene) is identical in both the normal and BF condition, obviating the value of traditional molecular markers as predictors of this disorder. The aberrant nature of such 'epigenetic' conditions have discouraged their research in mainstream genetics with most early studies limited to genetic disorders with dramatic economic consequences, such as almond BF and cherry crinkle and also some cancers in humans (**Figure 13**). Recent advances in our understanding of organismal genomics has shown that a diversity of epigenetic mechanisms exists which can play important roles in development. This realization has led to a research surge on epigenetic mechanisms, including the development of more accurate molecular-based diagnostics and possible treatments.

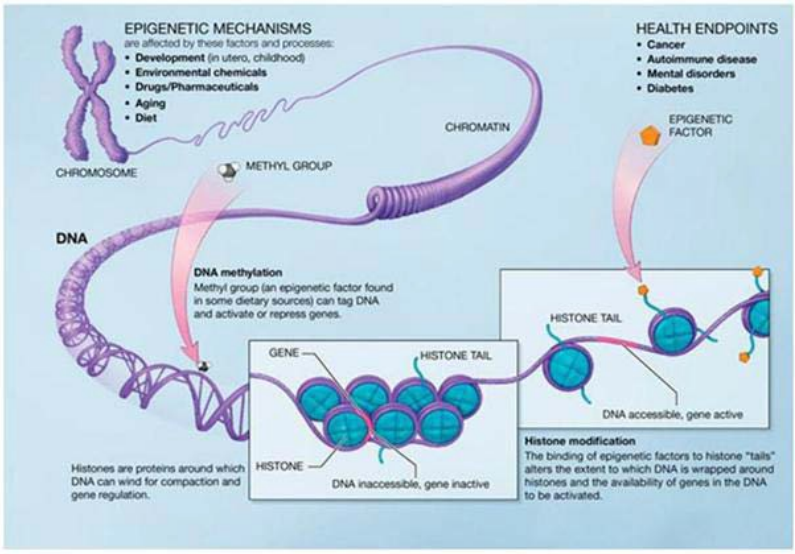


Figure 13. A summary of possible epigenetic mechanisms from more advanced human studies where control of a trait is determined not just by the simple presence or absence of a gene but rather by epigenetic mechanisms which act to enhance or suppress expression of genes.

For models based on standard Mendelian-gene control, a diverse array of molecular-based diagnostics is available (as summarized in literature citations 3, 4, 8, 14, 16, and 21). In this case the choice of molecular diagnostic would be made using standard marker assisted selection approaches such as PediMAP/Flex QTL software (see **Figure 12** and citation 8).

Initial field data, however, shows non-Mendelian segregation patterns, again supporting epigenetic control. Unlike Mendelian genetic control, where genes/traits are either present/absent, epigenetic mechanisms can vary in their degree of trait suppression resulting in varying levels of BF-phenotype. We are currently experimenting with specialized software (PediMAPand flex QT, L - see **Figure 9**) to develop the capacity to genetically characterized both discrete and variable-expression traits. The almond by peach UCD40A-17 tester progeny populations should be particularly useful in this process as the peach tester essentially allows us to identify the patterns of epigenetic inheritance in almond.

Appendix A. ELISA confirmation that BF-symptoms in Marcona are the result of infection by Prunus Necrotic Ringspot Virus (PNRSV; PDV – Prunus Dwarf Virus).

ELISA Testing for Marcona trees

	PNRSV		PDV
Marcona, tree BL7	positive	4/30/2010	negative
Marcona, tree DRT3	positive	4/30/2010	negative
Marcona, tree DRT4	positive	4/30/2010	negative
Marcona, tree DRT7	positive	4/30/2010	negative
Marcona, tree DRT11	negative	4/30/2010	negative
Marcona, tree DRT14	positive	4/30/2010	negative
Marcona, tree DRT18	positive	4/30/2010	negative

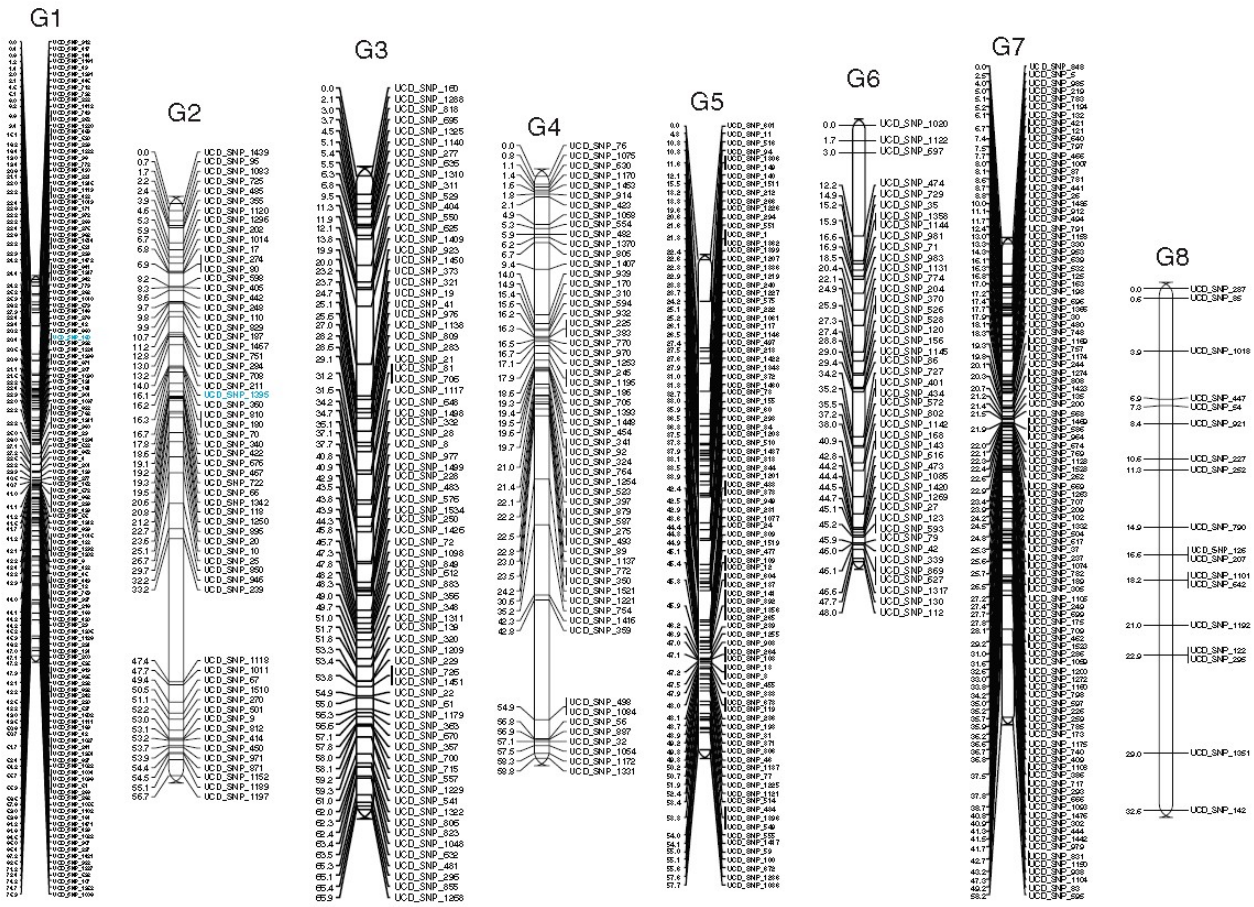
Appendix B. Molecular marker analysis verifying that the affected trees (Brown-Winters) are the Winters variety and not a propagation error.

NONPAREIL	182	194	130	148	142	146	211	233	99	110	212	259	224	236	155	155	148	158	151	167	115	121	134	152
PADRE	182	196	122	180	136	142	199	209	99	108	227	227	236	244	129	147	148	148	143	149	141	145	146	168
PRICE	194	196	146	148	130	148	199	211	110	114	212	227	224	236	129	155	148	148	167	167	121	141	134	166
RUBY	194	196	122	142	136	146	199	211	108	110	227	227	224	236	147	155	148	156	143	167	141	145	134	168
SOLANO	182	194	130	130	130	142	211	233	99	99	212	255	224	236	155	155	138	158						
SONORA	182	194	148	148	130	142	211	233	99	99	255	259	224	236	145	155	138	158	149	151	115	121	134	152
THOMPSON	194	216	122	130	130	142	203	211	99	114	212	227	224	236	147	155	136	148						
WINTERS	182	200	130	136	132	132	233	233	116	116	227	229	236	242	155	155	146	158	143	167	121	145	134	134
Brown-Winters	182	200	130	136	132	132	233	233	116	116	227	229	236	242	155	155	146	158	143	167	121	145	134	134

Appendix C. Yield performance of selections at the Billings Regional Variety Trials showing particularly high yields of Nonpareil clonal source (3-8-2-70) (from Bruce Lampinen 2011 RVT Annual Report)

				Kernel pounds per			
2011 Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	unit PAR int.	Tree	Acre	Cumulative kernel yield (lbs/acre)
Nonpareil-Nico	18776.9 a	0.99 bcde	68.0 abc	86.7 a	41.0 a	4964.2 a	19522.7 a
Nonpareil-3-8-2-70	17744.2 abc	1.05 bc	70.7 a	87.9 a	41.0 a	4962.3 a	18878.1 ab
Nonpareil-Newell	17790.9 abc	1.00 bcd	70.1 ab	81.0 ab	39.2 a	4744.7 a	18746.5 ab
Nonpareil-Driver	17943.0 ab	0.98 bcde	66.0 abcd	84.3 a	38.7 ab	4682.6 ab	18593.4 abc
Nonpareil-5	15744.6 de	1.03 bc	70.4 ab	78.0 ab	35.9 abc	4341.9 abc	17886.9 bcd
Nonpareil-6	16630.0 bcde	1.04 bc	70.0 ab	81.6 ab	38.1 ab	4618.5 ab	17838.3 bcd
2-19e	18253.3 ab	0.91 bcde	64.8 abcd	73.6 ab	36.8 ab	4459.7 ab	17560.0 bcd
Nonpareil-7	17078.8 abcd	0.83 e	69.2 abc	76.1 ab	31.4 bcd	3804.0 bcd	17235.0 cd
Nonpareil-Jones	16992.6 abcd	0.96 bcde	70.0 ab	81.6 ab	36.0 abc	4359.4 abc	17050.7 d
Winters	15979.0 cde	0.83 e	58.7 ef	76.3 bc	29.3 cde	3553.5 cde	14757.0 e
Chips	11900.6 f	0.94 bcde	60.3 de	51.4 de	24.6 de	2984.7 de	13917.8 e
Sweetheart	14969.2 e	0.86 de	64.1 bcde	52.5 de	28.2 de	3411.8 de	13712.5 e
Kahl	12420.0 f	0.89 cde	53.5 f	59.1 cd	24.4 de	2953.2 de	13514.3 e
Marcona	9633.4 g	1.07 b	30.8 g	51.8 de	22.7 e	2746.0 e	12053.7 f
Kochi	8701.4 g	1.22 a	63.5 cde	43.4 e	23.3 e	2825.2 e	11246.5 f

Appendix D. Almond by peach molecular marker map developed by our program (22). Molecular markers in almond by peach test progeny which may be found to be highly correlated with BF expression can then be used both as a marker or predictor of BF as well as a starting point to identify the specific gene(s) controlling this trait.



References Cited:

1. Arus, Pere, Thomas Gradziel, M. Margarida Oliveira, and Ryutaro Tao. 2009. Genomics of Almond. In K.M. Folta, S.E. Gardiner (eds.), Genetics and Genomics of Rosaceae, Springer Science. NY pp. 187-219.
2. Zhang, X. 2008. The Epigenetic Landscape of Plants. Science 25 April 2008: pg. 489-492.
3. Socias i Company R., O. Kodad, and J.M. Alonso and J.T.M. Gradziel. 2008. Almond Quality: A Breeding Perspective. In J. Janick (ed.) Horticultural Reviews. 34:197-238.
4. Gradziel, T.M. 2008. Almond (Prunus dulcis). In: M. Priyadarshan and S.M. Jain (eds).Breeding of Plantation crops. Springer Science publishers. pg. 1-33.
5. Granell, A., C. Pons, C. Martí, T.M. Gradziel, C.P. Peace, J. Forment and C. Royo E. Ogundiwin and C.H. Crisosto. 2007. Genomic Approaches - Innovative Tools to Improve Quality of Fresh Cut Produce. Acta Horticulturae 746:203-211.
6. Ogundiwin EA, Peace CP, Gradziel TM, Dandekar AM, Bliss FA, Crisosto CH (2007). Molecular genetic dissection of chilling injury in peach fruit. Acta Horticulturae 738:633-638.
7. Grant-Downton R.T. and H.G. Dickenson. Epigenetics and its Implications for Plant Biology 2. The 'Epigenetic Epiphany': Epigenetics, Evolution and Beyond. Ann. Bot., 2006; 97(1): 11 - 27.
8. Martínez-Gómez, P., Sánchez-Pérez, F., Dicenta, W. Howard and T.M., Gradziel. 2006. Almond. In: Kole C (ed) Genome Mapping & Molecular Breeding, Vol. 4, Fruits and Nuts, Chapt. 11, pg 229-242. Springer-Verlag, Heidelberg, Berlin.
9. Martinez-Gomez,-P; Sanchez-Perez, R; Vaknin, Y; Dicenta, F; Gradziel, TM. 2005. Improved technique for counting chromosomes in almond. Scientia-horticulturae. 105: 139-143.
10. Sanchez-Perez, R; Dicenta, F; Martinez-Gomez, P; Gradziel, TM. Origin of almond multiple embryos and potential utilization as near isogenic lines. Acta-horticulturae. 2004 Dec, no 663 (2); 819-822.
11. Martínez-Gómez, P., M. Rubio, F. Dicenta, and T.M. Gradziel.2004. Resistance to Plum Pox Virus (Dideron isolate RB3.30) in a group of California almonds and transfer of resistance to peach. J. Amer. Soc. Hort. Sci. 129(4):544-548.
12. Kester, D.E., K.A. Shackel, W.C. Micke, M. Viveros, and T.M. Gradziel. 2004. Noninfectious bud failure in 'Carmel' almond: I. Pattern of development in vegetative progeny trees. J. Amer. Soc. Hort. Sci. 129(2):244-249.
13. Martínez-Gómez, P., R. Sánchez-Pérez, F. Dicenta, and T.M. Gradziel. 2004. Rescue of multiple embryos in almond through in vivo micrografts. Food, Agriculture & Environment 2(2):273-275.
14. Ushijima, K., H. Sassa, A.M. Dandekar, T.M. Gradziel, R. Tao, and H. Hirano. 2003. Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed F-Box Gene with Haplotype-specific polymorphism. The Plant Cell 15(3):771-781.
15. Martínez-Gómez, P. and T.M. Gradziel. 2003. Sexual polyembryony in almond. Sexual polyembryony in almond. Sex Plant Reprod. 16:135-139.
16. Ushijima, K, Sassa H., Tamura, K. Kusaba, Tao R., Gradziel T.M., A.M. Dandekar and Hirano H. 2001. Characterization of the S-locus region of almond (Prunus dulcis): analysis of a somaclonal mutant and a cosmid contig for an S haplotype. Genetics 158:379-386.
17. Sathe, Shridhar K., Suzanne S. Teuber, Thomas M. Gradziel, and Kenneth H. Roux. 2001. Electrophoretic and immunological analyses of almond (*Prunus dulcis* L.) genotypes and

interspecies hybrids. J. Agric. Food Chem. 49(4):2043-2052.
18. Marcotrigiani, M. and T.M. Gradziel. 1997. Genetic mosaics and plant improvement. In J. Janick (ed.) Plant Breeding Reviews 15:43-84.
19. Kester, Dale E. and Thomas M. Gradziel. 1996. Genetic disorders, pp. 76-87. In: Micke, W.C. (ed.). Almond production manual. Publication 3364, California, Division of Agriculture and Natural Resources, Oakland, CA.
20. Kester, D.E., W.C. Micke, T.M. Gradziel, S. Arulsekar, K. Pelletreau, K. Shackel, M. Viveros, and R. Asay. 1994. Solving the problem of noninfectious bud-failure in California almond. Acta Horticulturae 373:35-39.
21. Dangl, Gerald S., Judy Yang, Thomas Gradziel, Deborah A. Golino. 2009. A practical method for almond cultivar identification and parental analysis using simple sequence repeat markers. Euphytica 168:41-48.
22. Riaz Ahmad, Dan E. Parfitt, Joseph Fass, Ebenezer Ogundiwin, Amit Dhingra, Thomas M. Gradziel, Dawei Lin, Nikhil A. Joshi, Pedro J. Martinez-Garcia, Carlos H. Crisosto. 2012. Whole genome sequencing of peach (Prunus persica L.) for SNP identification and selection. BMC Genomics 2011, 12:569 doi:10.1186/1471-2164-12-569.

Research Effort Recent Publications:

Sorkheh K., B. Shiran, M. Khodambashi, H. Moradi, T.M. Gradziel, P. Martinez-Gomez. 2010. Correlations between quantitative tree and fruit almond traits and their implications for breeding. Scientia Horticulturae 125:323–331.

Cantin, C. M. Crisosto, C. H. Ogundiwin, E. A. Gradziel, T. Torrents, J. Moreno, M. A. Gogorcena, Y. 2010. Chilling injury susceptibility in an intra-specific peach [Prunus persica (L.) Batsch] progeny. Postharvest Biology and Technology. 58:79-87.

Zeinalabedini, M. Khayam-Nekoui, M. Grigorian, V. Gradziel, T. M. Martinez-Gomez, P. 2010. The origin and dissemination of the cultivated almond as determined by nuclear and chloroplast SSR marker analysis. Scientia Horticulturae 125: 593-601.

Rahemi A., R. Fatahi, A. Ebadi, T. Taghavi, D. Hassani, T. Gradziel, and J. Chaparro. 2010. Genetic variation of S-alleles in wild almonds and their related Prunus species. Australian Journal of Crop Science 4:648-659.

Sorkheh, K., Shiran, B., Asadi, E., Jahanbazei, H., Gradzeil, T. M., Mohammady, SH 2011. Evaluation of Genetic Variation, Relatedness, and Testing The Genetic Origin of Cultivated Genotypes and Wild Species of Almond using AFLP Markers, Scientific Journal of Agriculture.

Sorkheh, K., B. Shiran, R. Ahmad, P. Martinez-Gomez, T.M. Gradziel, N.Amirbakhtiar 2011. Analysis of Almond [Prunus dulcis (Miller) D. A. Webb] cultivars and related wild species using SSR and AFLP markers. Journal of Horticultural and biotechnology.

Sánchez-Pérez R, M Zeinalabedini, M. Khayam-Nekoui, V Grigorian, T.M. Gradziel, and P Martínez-Gómez. 2011. The origin and dissemination of the cultivated almond as determined by nuclear and chloroplast SSR marker analysis. Scientia Horticulturae.

Shiran, B. Sorkheh, K., V. Rouhi, T. M. Gradziel, B. K. Epperson, P. Martinez-Gomez 2011. Molecular characterization of Iranian almond cultivars and related wild species using amplified fragment-length polymorphisms (AFLPs). Zaragoza (Spain), 16-20 September. Acta Horticulture.

Hamby, K., L.W. Gao, B. Lampinen, T. Gradziel and F. Zalom. 2011. Hull Split Date and Shell

Seal in Relation to Navel Orangeworm (Lepidoptera: Pyralidae) Infestation of Almonds. Hort. Entom.: 104-965-969.
Granell, A., Pons, C., Martí, C., Forment, J., Royo, C., Gradziel, T.M., Peace, C.P., Ogundiwin, E. and Crisosto, C.H. 2011. Genomic approaches – innovative tools to improve quality of fresh cut produce. *Acta Hort.* 746:203-212.
Gradziel, T.M. 2011. Almond origin and domestication. In J. Janick (ed.) Horticultural Reviews. 38:23-82.
Socias i Company, R., J.M. Alonso, O. Kodad and T.M. Gradziel. 2011. Almonds. In: M.L. Badenes and D.H. Byrne (eds.), Fruit Breeding, Handbook of Plant Breeding 8. Springer N.Y. pg. 697-728.
Gradziel, T.M. 2012. Classical genetics and traditional breeding. In: A. G. Abbott & C. Kole (eds.). Genetics, Genoics and Breeding of Stone Fruits. Science Publishers, Inc., Plymouth. pg. 1-50.
Riaz Ahmad, Dan E. Parfitt, Joseph Fass, Ebenezer Ogundiwin, Amit Dhingra, Thomas M. Gradziel, Dawei Lin, Nikhil A. Joshi, Pedro J. Martinez-Garcia, Carlos H. Crisosto. 2012. Whole genome sequencing of peach (Prunus persica L.) for SNP identification and selection. BMC Genomics 2011, 12:569 doi:10.1186/1471-2164-12-569.
Martínez-García P., Peace C., Parfitt D., Ogundiwin E., Fresnedo-Ramírez J., Dandekar A., Gradziel T., Crisosto C. 2012 Influence of year and genetic factors on chilling injury susceptibility in peach (*Prunus persica* (L. Batsch). Euphytica (Online first): 1-14. DOI: 10.1007/s10681-011-0572-1.
Frett, T., K. Kasic, J. Clark, D. Byrne, T. Gradziel and C. Crisosto. In-press. Standardized phenotyping for fruit quality in peach [Prunus persica (L.) Batsch]. AortScience